

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Stomp et al.  
Serial No.: To be assigned  
Filed: Concurrently herewith  
For: *GENETICALLY ENGINEERED DUCKWEED*

Date: October 4, 2001

Box Patent Application  
Commissioner for Patents  
Washington, DC 20231

**SUBMISSION OF SUBSTITUTE SPECIFICATION  
FOR PUBLICATION UNDER 35 U.S.C. § 122 (B)**

Sir:

Applicants are filing concurrently herewith a continuing application under 37 C.F.R. § 1.53(b). Accordingly, Applicants are submitting the enclosed substitute specification containing the new claim set and priority claim. Applicants submit that the substitute specification contains no new matter as compared with the originally filed specification.

It is respectfully requested that the substitute specification, containing the claims presented for prosecution in the current application be published under 35 U.S.C. § 122 (b) instead of the originally filed specification.

The substitute specification contains the following amendments to the specification and claims:

**Amendment**

Please cancel Claims 1-37, 39 and 49-60, which were already prosecuted in the parent application or are drawn to non-elected inventions.

**In the Specification.**

Please amend the specification on page 1, line 4, before the Field of the Invention to add the following as a new paragraph:

TOP SECRET - USPTO

## RELATED APPLICATION INFORMATION

This application is a continuation of U.S. Patent Application Serial No. 09/448,105, filed November 23, 1999, which is a divisional of U.S. Patent Application Serial No. 09/132,536 filed 11 August 1998, issued as U.S. Patent No. 6,040,498, which claims the benefit of U.S. Provisional Application No. 60/055,474 filed 12 August 1997, the disclosures of which are incorporated by reference herein in their entireties.

### In the Claims.

Please amend the claims as follows:

38. (Amended) A stably transformed duckweed plant comprising a heterologous nucleic acid of interest incorporated in its genome.
40. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said duckweed plant comprises fewer than 5 copies of said heterologous nucleic acid of interest.
41. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said duckweed plant is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolfiella*, and genus *Lemna*.
42. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said duckweed plant is selected from the genus *Lemna*.
43. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said duckweed plant is selected from the group

- consisting of a species of *Lemna minor*, a species of *Lemna miniscula*, and a species of *Lemna gibba*.
44. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said nucleic acid comprises at least one expression cassette comprising a gene which confers resistance to a selection agent.
45. (Amended) The stably transformed duckweed plant according to Claim 44, wherein said gene which confers resistance to a selection agent is selected from the group consisting of *neo*, *bar*, *pat*, *ALS*, *HPH*, *HYG*, *EPSP* and *Hml*.
46. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said nucleic acid comprises two genes of interest.
47. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said nucleic acid encodes a protein or peptide selected from the group consisting of insulin, growth hormone,  $\alpha$ -interferon,  $\beta$ -glucocerebrosidase, retinoblastoma protein, p53 protein, angiostatin, leptin, and serum albumin.
48. (Amended) The stably transformed duckweed plant according to Claim 38, wherein said nucleic acid encodes at least one protein or peptide subunit of a multimeric protein selected from the group consisting of hemoglobin, collagen, P450 oxidase, and a monoclonal antibody.

Please add the following new claims:

61. The stably transformed duckweed plant according to Claim 38, wherein said nucleic acid encodes a secreted protein or peptide.

62. The stably transformed duckweed plant according to Claim 43, wherein said duckweed plant is from a species of *Lemna minor*.
63. A stably transformed duckweed plant tissue comprising a heterologous nucleic acid of interest incorporated in its genome.
64. The stably transformed duckweed plant tissue according to Claim 63, wherein said plant tissue is meristematic tissue.
65. The stably transformed duckweed plant tissue according to Claim 63, wherein said plant tissue is frond tissue.
66. The stably transformed duckweed plant tissue according to Claim 63, wherein said plant tissue is callus tissue.
67. The stably transformed duckweed plant tissue according to Claim 66, wherein said plant tissue is Type I callus tissue.
68. A duckweed tissue culture comprising the stably transformed duckweed plant tissue of Claim 63.
69. A stably transformed duckweed cell comprising a heterologous nucleic acid of interest incorporated in its genome.
70. A stably transformed duckweed plant comprising a chimeric nucleic acid of interest incorporated in its genome, wherein said chimeric nucleic acid comprises a coding sequence operably linked to a transcription initiation region that is heterologous to said coding sequence.

71. The stably transformed duckweed plant according to Claim 70, wherein said chimeric nucleic acid comprises a duckweed coding sequence operably linked to a transcription initiation region that is heterologous to said coding sequence.
72. The stably transformed duckweed plant accordingly to Claim 70, wherein said chimeric nucleic acid is flanked by T-DNA border sequences.
73. The stably transformed duckweed plant according to Claim 70, wherein said duckweed plant comprises fewer than 5 copies of said chimeric nucleic acid.
74. The stably transformed duckweed plant according to Claim 70, wherein said duckweed plant is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolfiella*, and genus *Lemna*.
75. The stably transformed duckweed plant according to Claim 70, wherein said duckweed plant is selected from the genus *Lemna*.
76. The stably transformed duckweed plant according to Claim 70, wherein said duckweed plant is selected from the group consisting of a species of *Lemna minor*, a species of *Lemna miniscula*, and a species of *Lemna gibba*.
77. The stably transformed duckweed plant according to Claim 38, wherein said chimeric nucleic acid of interest comprises at least one expression cassette comprising a gene which confers resistance to a selection agent.

78. The stably transformed duckweed plant according to Claim 77, wherein said gene which confers resistance to a selection agent is selected from the group consisting of *neo*, *bar*, *pat*, *ALS*, *HPH*, *HYG*, *EPSP* and *Hml*.
  79. The stably transformed duckweed plant according to Claim 70, wherein said chimeric nucleic acid comprises two genes of interest.
  80. The stably transformed duckweed plant according to Claim 70, wherein said chimeric nucleic acid encodes a protein or peptide selected from the group consisting of insulin, growth hormone,  $\alpha$ -interferon,  $\beta$ -glucocerebrosidase, retinoblastoma protein, p53 protein, angiostatin, leptin, and serum albumin.
  81. The stably transformed duckweed plant according to Claim 70, wherein said chimeric nucleic acid encodes at least one protein or peptide subunit of a multimeric protein selected from the group consisting of hemoglobin, collagen, P450 oxidase, and a monoclonal antibody.
  82. The stably transformed duckweed plant according to Claim 70, wherein said chimeric nucleic acid encodes a secreted protein or peptide.
  83. The stably transformed duckweed plant according to Claim 76, wherein said duckweed plant is from a species of *Lemna minor*.
  84. A stably transformed duckweed plant tissue comprising a chimeric nucleic acid of interest incorporated in its genome, wherein said chimeric nucleic acid comprises a coding sequence operably linked to a transcription initiation region that is heterologous to said coding sequence.

85. The stably transformed duckweed plant tissue according to Claim 84, wherein said plant tissue is meristematic tissue.
  86. The stably transformed duckweed plant tissue according to Claim 84, wherein said plant tissue is frond tissue.
  87. The stably transformed duckweed plant tissue according to Claim 84, wherein said plant tissue is callus tissue.
  88. The stably transformed duckweed plant tissue according to Claim 87, wherein said plant tissue is Type I callus tissue.
  89. A duckweed tissue culture comprising the stably transformed duckweed plant tissue of Claim 84.
  90. A stably transformed duckweed cell comprising a chimeric nucleic acid of interest incorporated in its genome, wherein said chimeric nucleic acid comprises a coding sequence operably linked to a transcription initiation region that is heterologous to said coding sequence.

**Remarks**

Claims 38, 40-48 and 61-90 are pending in this application following entry of the foregoing preliminary amendment. The marked up version of the claims is attached hereto and is entitled "**Version with Markings to Show Changes Made**".

Support in the specification for the claim amendments presented above is discussed below.

Claims 38 and 40-48 have been amended to recite a "stably" transformed duckweed plant. Likewise, new Claims 61-90 recite a "stably" transformed duckweed plant, tissue or cell. This recitation is supported throughout the specification, for example, in the Examples and at page 3 (lines 19-21), which states: "The methods involve the use of ballistic bombardment, *Agrobacterium*, or electroporation to stably introduce and express a nucleotide sequence of interest in transformed plants."

Claims 40-44 and 46-48 have been amended to depend from Claim 38 rather than canceled Claim 39.

Claims 43 and 45 have been amended to correct minor clerical errors and to properly recite a Markush group.

New Claim 61 recites a heterologous nucleotide sequence that encodes "a secreted protein or peptide." This claim is supported by the specification, for example, at page 8 (lines 19-20), which recites: "In particular preferred embodiments, the nucleic acid encodes a secreted protein or peptide."

New Claim 62 specifically recites that the duckweed plant is a species of *Lemna minor*. This claim is supported by the Markush group of Claim 43 from which Claim 62 depends.

New independent Claim 63 recites a "stably transformed duckweed plant tissue." This claim is similar to Claim 38, except that new Claim 63 recites a "duckweed plant tissue" rather than "duckweed plant" as recited by Claim 38. New Claim 63 is supported throughout the specification, including for example, at page 3 (lines 22-23), which states: "Transformed duckweed

PCT/US2014/042680

cells, tissues, plants and seed are also provided." The specification further states at page 7 (lines 8-9): "Any suitable duckweed cell or tissue type can be transformed according to the present invention."

New dependent Claims 64-67 specifically recite that the transformed plant tissue is meristematic tissue, frond tissue, callus tissue, or Type I callus tissue. These claims are supported by the specification at page 16 (lines 6-8), which recites: "The methods of the invention are useful for transforming duckweed plant cells, preferably frond and meristematic cells. Such cells also include callus, which can be originated from any tissue of duckweed plants." The specification further states at page 16 (lines 27-28), "Preferably, Type I or Type III callus, more preferably Type I callus, is used to transform duckweed according to the present invention."

New Claim 68 recites a "duckweed tissue culture comprising the stably transformed duckweed plant tissue of Claims 63." This claim is supported throughout the specification, for example, at page 4 (lines 10-12) which states: "As a fourth aspect, the present invention provides transformed duckweed plants and transformed duckweed tissue culture produced by the methods described above."

New independent Claim 69 recites a "stably transformed duckweed cell." This claim is similar to Claim 38, except that new Claim 69 recites a "duckweed cell" rather than "duckweed plant" as recited by Claim 38. New Claim 69 is supported throughout the specification, including for example, at page 3 (lines 22-23), which states: "Transformed duckweed cells, tissues, plants and seed are also provided." The specification further states at page 7 (lines 8-9): "Any suitable duckweed cell or tissue type can be transformed according to the present invention."

New Claims 70 and 73-90 are similar to Claims 38, 40-48 and 61-69, except that Claims 70 and 73-90 recite a stably transformed duckweed plant, tissue or cell "comprising a chimeric nucleic acid of interest incorporated in its genome, wherein said chimeric nucleic acid comprises a coding sequence operably linked to a transcription initiation region that is heterologous to said

In re: Stomp et al.  
Serial No.: To be assigned  
Filed: Concurrently herewith  
Page 10 of 12

coding sequence." This recitation is supported by the specification as filed, for example at page 10, lines 20-22, which recites: "As used herein a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence."

New Claim 71 specifically recites that the coding sequence is a "duckweed coding sequence." This recitation is supported by the specification, e.g., at page 8 (lines 15-16), which recites; "The nucleic acid may be from duckweed or from another organism (*i.e.*, heterologous)."

New Claim 72 is similar to originally filed Claim 39, except that it depends from Claim 70 instead of Claim 38.

In the view of the foregoing, Applicants submit that the amendments to the claims present no new matter, and respectfully request entry thereof.

**Conclusion.**

Claims 38, 40-48 and 61-90 are pending in this application. Applicants submit that this application is in condition for substantive examination, which action is respectfully requested.

Respectfully submitted,

  
Karen A. Magri

**Customer Number:**



20792

PATENT TRADEMARK OFFICE

**CERTIFICATE OF EXPRESS MAILING**

Express Mail Label No. EL733099585US

Date of Deposit: October 4, 2001

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box Patent Application, Commissioner For Patents, Washington, DC 20231.



Traci A. Brown

Date of Signature: October 4, 2001

**Version with Markings to Show Changes Made**

38. (Amended) A stably transformed duckweed plant comprising a heterologous nucleic acid of interest incorporated in its genome.
40. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said duckweed plant comprises fewer than 5 copies of said heterologous nucleic acid of interest.
41. (Amended) The stably transformed duckweed plant according to Claim 38 [or Claim 39], wherein said duckweed plant is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolfiella*, and genus *Lemna*.
42. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said duckweed plant is selected from the genus *Lemna*.
43. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said duckweed plant is selected from the group consisting of a species of *Lemna minor*, a species of *Lemna miniscula*, and a species of *Lemna gibba*.
44. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said nucleic acid comprises at least one expression cassette comprising a gene which confers resistance to a selection agent.
45. (Amended) The stably transformed duckweed plant according to Claim 44, wherein said gene which confers resistance to a selection agent is selected from the group consisting of *neo*, *bar*, *pat*, *ALS*, *HPH*, *HYG*, *EPSP* and *Hml*.

46. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said nucleic acid comprises two genes of interest.
47. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said nucleic acid encodes a protein or peptide selected from the group consisting of insulin, growth hormone,  $\alpha$ -interferon,  $\beta$ -glucocerebrosidase, retinoblastoma protein, p53 protein, angiostatin, leptin, and serum albumin.
48. (Amended) The stably transformed duckweed plant according to Claim 38 [39], wherein said nucleic acid encodes at least one protein or peptide subunit of a multimeric protein selected from the group consisting of hemoglobin, collagen, P450 oxidase, and a monoclonal antibody.

\*\*\*\*